AWARD NUMBER: W81XWH-16-1-0234

TITLE: Exploiting RhoA Mutations in Diffuse Gastric Adenocarcinoma and Targeting Intertwined RhoA and Yap1 Pathways for Therapeutic Advantage

PRINCIPAL INVESTIGATOR: Dr. Jaffer Ajani

CONTRACTING ORGANIZATION: The University of Texas MD Anderson Cancer Center
Houston, TX 77030

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
October 2017

2. REPORT TYPE
Annual

3. DATES COVERED
15 Sep 2016 - 14 Sep 2017

4. TITLE AND SUBTITLE

Exploiting RhoA Mutations in Diffuse Gastric Adenocarcinoma and Targeting Intertwined RhoA and Yap1 Pathways for Therapeutic Advantage

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0234

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Jaffer Ajani

E-Mail: jajani@mdanderson.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Texas MD Anderson Cancer Center
1515 Holcombe Blvd Unit 207
Houston, TX 77030-4017

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Our IDEA is on the right track.

We have successfully inserted a fluorescent marker mOrange into MIT's Dr. Zhang's pLenti-Crispr-v2, making transfection into mammalian cells easier and visible under fluorescent microscope, it the same time, those cells under Crispr editing are also selectable with puromycin.

We have successfully knocked-out RhoA expression in cell lines of AGS and GT5.

With RhoA knockout in cell line GT5, Yap1 is also significantly downregulated.

Reintroducing mutant *RhoA*-Y42C is in progress.

15. SUBJECT TERMS
RHOA, YAP1, mouse model, CRISPR-CAS9, plasmid, cell lines, diffuse gastric adenocarcinoma, mutations, gastric adenocarcinoma

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	34	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The goal of this Idea project is to identify hotspot RHOA mutations in gastric adenocarcinoma (cell lines and patient-derived specimens). Carry out functional studies (genomic manipulations) as they relate to RHOA mutations and demonstrate that RHOA mutation regulates YAP1 activity. Finally, to develop a genetic mouse model by introducing mutant RHOA.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

RHOA, YAP1, mouse model, CRISPR-CAS9, plasmid, cell lines, diffuse gastric adenocarcinoma, mutations, gastric adenocarcinoma

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1: Identification of overexpression and/or activating mutations of RhoA in novel cancer models of GAC (patient derived primary tumor cells and GAS TMA) to determine their clinical relevance and relationship to Yap1 activation.

Aim 2: Elucidate oncogenic functions of WT/mutant RhoA in GAC cells and determine if WT or mutant RhoA activates Hippo pathway coactivator Yap1 by assessing nuclear accumulation and transcriptional activity (bidirectional) in GAC cells.

Aim 3: Develop a genetically engineered mouse model of RhoA mutation and Yap1 expression in stomach and assess the effects of RhoA inhibitor or in combination of Yap1 inhibition in vitro and in vivo to set the stage for a clinical trial in dGAC patients.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

DOD Progress report

Principal Investigator: Jaffer A. Ajani.

GAC is rampant around the world with nearly 1 million new cases per year globally. Majority of GAC patients are diagnosed at an advanced stage and have a median survival of ~10 months. The purpose of this application is to establish if mutated *RhoA* signaling activates Yap1 signaling (Yap1 being the tumor resistance highway for many oncogenic pathways in diffuse GACs [dGACs]). We are to explore the functional significance and inter-dependence of both RhoA and Yap1 pathways (including in a *RhoA* mutant mouse model).

Rationale: Important efforts including TCGA, Cosmic, and others researchers have uncovered recurrent RhoA mutations that are uniquely observed in dGACs. RhoA mutations are common (described in as many as 25% of dGACs) and the pattern is unique (GTG>GGG or CTT>CGT, or TCT>TGT). RhoA mutations often lead to gain-of-function. RhoA has 3 mutational hotspots in the functional domains (GTP-binding site, effector-binding site, and regions specific to ROCK1, etc.). In the Cosmic database, the major 3 substitutional mutations are R5Q, G17V and Y42C. We are focused on RhoA signaling that most likely activates Yap1, a key signal in Hippo pathway.

Since the award, we have completed considerable volume of work towards what we proposed as an IDEA grant. We summarize our progress made:

1. **Identification of mutations of *RhoA* and overexpression of YAP1 in patient-derived GAC cells and GAC cell lines.**

<u>1.1 To detect point mutations in GAC cell lines.</u> To study the functions of *RhoA* mutations, we studied 9 GAC cell lines (Snu16, AGS, GT5, Kato3, MKN45, N87, Snu1, Snu5, and YCC1). Genomic DNA was extracted and sequenced. Established sequences for Exon2 from PCR products using RhoA primers hRhoA.F3.M13F 5’ GTAAAACGACGGCCAGaggtggatcggcgtactaga 3’hRhoA.R3.M13R 5' TCACACAGGAAACAGCTATGACacatgcctgtaatccctgct 3'.

Results are shown below that out of 9 GC cell lines sequenced for Exon2, Snu16 is found with a F39L point mutation.

Snu16 F39L
RhoA-WT ccagttcccagaggtgtatgtgcccacagtgtttgagaactatgtggcagatatcgaggt 240
Snu16-M13F CCAGTTCCCAGAGGTGTATGTGCCCACAGTGTTGGAGAACTATGT GGCAGATATCGAGGT 170
AGS-M13F CCAGTTCCCAGAGGTGTATGTGCCCACAGTGTTTGAGAACTATGT GGCAGATATCGAGGT 169
GT5-M13F CCAGTTCCCAGAGGTGTATGTGCCCACAGTGTTTGAGAACTATGT GGCAGATATCGAGGT 170

```
Kato3-M13F     CCAGTTCCCAGAGGTGTATGTGCCCACAGTGTTTGAGAACTATGT
GGCAGATATCGAGGT  170
MKN45-M13F
CCAGTTCCCAGAGGTGTATGTGCCCACAGTGTTTGAGAACTATGT
GGCAGATATCGAGGT  170
N87-M13F       CCAGTTCCCAGAGGTGTATGTGCCCACAGTGTTTGAGAACTATGT
GGCAGATATCGAGGT  170
Snu1-M13F      CCAGTTCCCAGAGGTGTATGTGCCCACAGTGTTTGAGAACTATGT
GGCAGATATCGAGGT  170
Snu5-M13F      CCAGTTCCCAGAGGTGTATGTGCCCACAGTGTTTGAGAACTATGT
GGCAGATATCGAGGT  170
YCC1-M13F
CCAGTTCCCAGAGGTGTATGTGCCCACAGTGTTTGAGAACTATGT
GGCAGATATCGAGGT  170
          ********************************* **************************
```

In addition to the PCR products of *RhoA*, cDNAs from 9 GC cell lines were cloned into pGEM vector, and then sequenced for Exon2, two more cell lines were found containing *RhoA* mutation such as Snu1 is found with a K6N point mutation and Kato III is found E64- deletion.

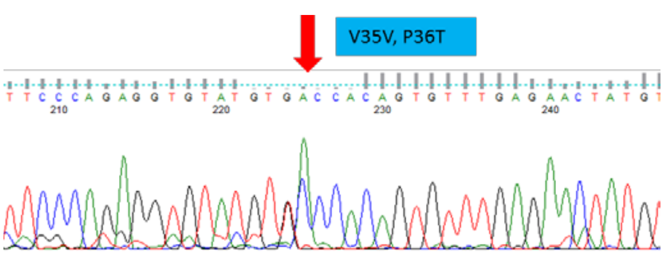
```
                                                              Kpn1      Snu1-1 solid C, K6N
hRhoA2-Kato.1       -------------------GGGATTAAGGTACCATGGCTGCCATCCGGAAGAAACTGGTG 41
hRhoA2-Snu1.2       --------------------GGATTAAGGTACCATGGCTGCCATCCGGAAGAAACTGGtG 40
hRhoA2-Ycc2.2       ---------GGCCGC----GGGATTAAGGTACCATGGCTGCCATCCGGAAGAAACTGGTG 47
hRhoA2-Kato.3       ---------GGCCGC----GGGATTAAGGTACCATGGCTGCCATCCGGAAGAAACTGGTG 47
hRhoA2-GT5.2        ---CGCCATGGCCGC----GGGATTAAGGTACCATGGCTGCCATCCGGAAGAAACTGGTG 53
hRhoA2-Snu1.1r      CCTGCAGGCGGCCGCACTAGTGATTAAGGTACCATGGCTGCCATCCGGAACAAACTGGTG 224
hRhoA2-Ycc2.1r      CCTGCAGGCGGCCGCACTAGTGATTAAGGTACCATGGCTGCCATCCGGAAGAAACTGGTG 224
hRhoA2-Kato.2r      CCTGCAGGCGGCCGCACTAGTGATTAAGGTACCATGGCTGCCATCCGGAAGAAACTGGTG 239
hRhoA2-GT5.3r       CCTGCAGGCGGCCGCACTAGTGATTAAGGTACCATGGCTGCCATCCGGAAGAAACTGGTG 214
hRhoA-NM1664        ACT---------CGGATTCGTTGCCTGAGCA--ATGGCTGCCATCCGGAAGAAACTGGTG 127
                                                * *   ***************** *********
```

```
hRhoA2-Kato.1
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAG---
GATTATGATCGCCTG 218
hRhoA2-Snu1.2
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAGGA
AGATTATGATCGCCTG 220
hRhoA2-Ycc2.2
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAGGA
AGATTATGATCGCCTG 227
hRhoA2-Kato.3
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAGGA
AGATTATGATCGCCTG 227
```

hRhoA2-GT5.2
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAGGA
AGATTATGATCGCCTG 233
hRhoA2-Snu1.1r
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAGGA
AGATTATGATCGCCTG 404
hRhoA2-Ycc2.1r
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAGGA
AGATTATGATCGCCTG 404
hRhoA2-Kato.2r
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAG---
GATTATGATCGCCTG 416
hRhoA2-GT5.3r
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAGGA
AGATTATGATCGCCTG 394
hRhoA-NM1664
GGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGGCAGGA
AGATTATGATCGCCTG 307
****
****
Kato solid E64-

hRhoA.Wt.F.Kpn1 5' aaGGTACCATGGCTGCCATCCGGAAGAAACTGGTGATTGTTGGTGATG 3'
hRhoA.Wt.R.Xba1 5' aaTCTAGActCAAGACAAGGCACCCAGATTTTTTCTTCCCACG 3'
Sequencing primer, T7.F 5´- TAATACGACTCACTATAGGG- 3´ after RhoA Exon2 is cloned into pGEM vector.

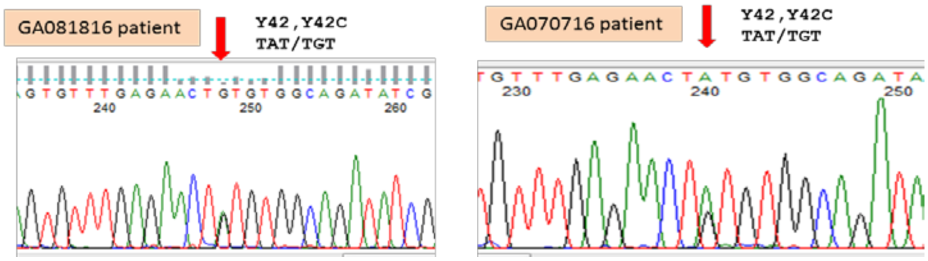
In addition, we also found a GE-Junction adenocarcinoma cell line OE19 also has *RhoA* mutation in exon 2 of *RhoA* as a SNP of V35V silent mutation and P36T is detected as following.



In summary, a total 10 cell lines were subjected to genomic DNA and cDNA sequencing, we found RhoA mutations in **four** GC cell lines. These were cell line Snu1 with F6N point mutation; cell line Snu16 with F39L point mutation; cell line Kato III with a E64- deletion; and cell line OE19 with a V35V and P36T mutations.

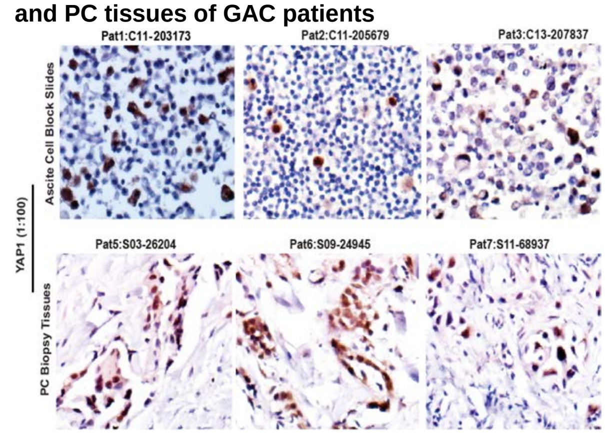
1.2 <u>Identification of hotspot mutation Y42C of RhoA in patient-derived GAC cells</u>

Patient-derived tumor cells are a precious resource to identify targetable drivers which can be translated into the clinics. Thus, we collected GAC patients' malignant ascites cells and isolate their genomic DNA and total RNA which reversely transcript to cDNA. We then amplified *RhoA* Exon2 in these cells. In all 18 patients samples, PCR product of Exon2 of *RhoA* were amplified, purified and then sequenced. We found the hotspot Y42C mutations in two patients. One is GA081816 and another one is GA070716 as shown below. Thus we found that 11% of GAC patient-derived cells were had hotspot Y42C mutations.



1-3. YAP1 is highly upregulated in malignant ascites cells and metastatic GAC tissues. The Hippo pathway and its transcriptional coactivator Yes-associated protein (YAP1) has emerged as major regulators of organ size and proliferation [{Tumaneng, 2012 #8;Tumaneng, 2012 #750;Tumaneng, 2012 #750}]. YAP1 overexpression and its activation (nuclear localization) correlate with poor outcome in several tumor types.[{Xu, 2009 #21550;Kang, 2011 #20650;Lee, 2016 #1063}] To understand the expression and function of YAP1 in peritoneal carcinomatosis (PC; which often due to dGAC), we first detected YAP1 level in 24 patients ascites cell block and 22 additional specimens derived from patient biopsies from PC. Fig. xx demonstrates that YAP1 is highly expressed only in malignant cells in both ascites cell block FFPE slide (Fig. 1; top panel) and biopsy of PC tissues (Fig.1; lower panel) but not in other cell types (immune or stromal cells etc.). YAP1 is highly expressed in PC and could potentially be a tumor marker and target of PC. Our idea was that *RhoA* mutation could activate YAP1.

Figure 1. YAP1 Marks malignant cells in ascite cells and PC tissues of GAC patients

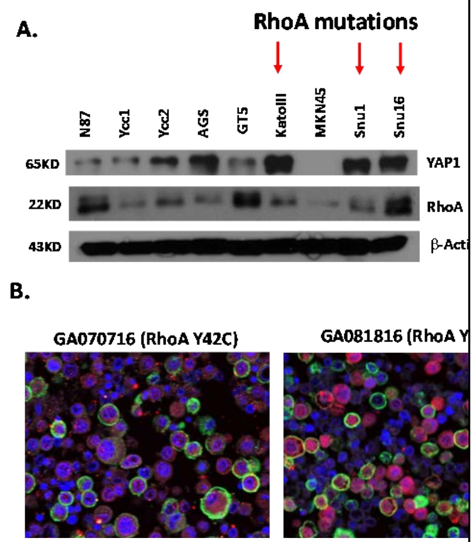


1-4. Overexpression of YAP1 correlates with *RhoA* mutated GAC cell lines and patient-derived GAC cells. To investigate if mutations of *RhoA* is associated with YAP1 expression, we first detect YAP1 in GAC cell lines with *RhoA* mutants (SNU1, SNU16 and Kato III) or WT ones. We found that 3 *RhoA* mutated GAC cell lines-Snu1, kato III and Snu16 have relative high YAP1 expression (Figure 2). Furthermore, there is higher YAP1 expression in GA081816 and GA070716 patient-derived cells with *RhoA* Y42C mutations than that with WT type ones (Figure 2) indicating that *RhoA* mutations may activate YAP1 expression.

2. Elucidate oncogenic functions of WT/mutant *RhoA* in GAC cells and determine if WT or mutant *RhoA* activates Hippo pathway coactivator Yap1 in GAC cells.

<u>2-1. *Establish RhoA deleted GAC cells.*</u> To study the functional consequences of *RhoA* alterations including mutation and deletion, we planned to knock-out Y42 Wt *RhoA* coding protein, and replace with Y42C hotspot point mutation in GAC cell lines and study its downstream biological effects. All available GC cell lines have RhoA overexpressed (this means that RhoA is ubiquitously expressed and by expression, one cannot tell if the protein is mutated or not. In other words, the currently available antibody cannot distinguish between mutated and non-mutated protein. This is one reason; we did not perform RhoA expression in our TMA). We first knocked down *RhoA* using a modified lentiviral plasmid, pCrispr-V2 (Addgene plasmid) with addition of mOrange, which would render Cas9-guide RNA expressed cells fluorescent for visibility and capable of cell sorting. Coding mOrange sequence is inserted in-frame with existing Cas9 and Puro cDNAs. Three coding genes of *Cas9*, *mOrange* and *Puromycin* are linked with P2A and T2A respectively.

Figure 2. *RhoA* mutations are associated with YAP1 overexpression in GAC cell lines and patients derived ascites cells. A. Expression of YAP1 in GAC cell lines by western blot; **B.** Immunofluorecent staining of YAP1 (red) and EpCam (green) in two patients' derived cells with RhoA mutations (Y42C)



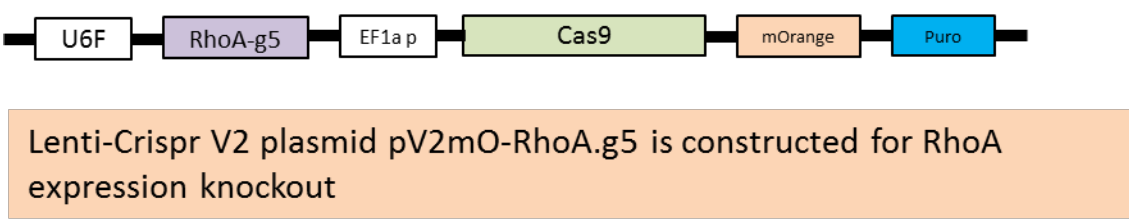
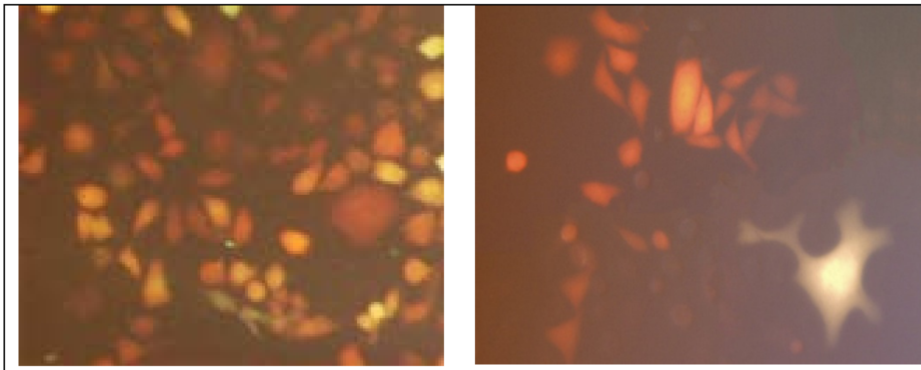


Figure 3. A *RhoA* guide-RNA at location in vicinity of Y42 of the *RhoA* Exon2 is designed in such a way that re-introduction of mutant Y42 expression would not be re-cut by integrated Cas9-RhoA.g5 Crispr enzyme. RhoA.guideRNA5 primers formed duplex and then is cloned into pLentiCrispr-V2.

As shown in Figure 4, we established *RhoA* knock out in AGS and GT-5 cells with mOrange positive selected by flowcytometry and confirmed that these cells are with knock out of *RhoA* by western blot as show in Figure 5. We also found that knock down *RhoA* in both AGS and GT-5 greatly reduced YAP1 expression indicating RhoA is upstream YAP1 and regulate YAP1 expression.



AGS expressing RhoA-gRNA5 and Cas9-mOrange-Puromycin, flow-cytometry sorted cells.

GT5 expressing RhoA-gRNA5 and Cas9-mOrange-Puromycin, flow-cytometry sorted cells.

Figure 4. Co-transfect guide RNA RhoA U6F-gRNA5 and above construct EF1a-prom-Cas9-mOrange-Puro, transfected cells are sorted by flow cytometry, those expressing mOrange and Puro-resistant cells are presumed *RhoA*-KO cells.

2-2. *Down regulation of RhoA in GAC cells reduced YAP1 expression* We have transfected EF1a-prom-Cas9-mOrange-Puro into 293T cells and harvest Lentivirus infect AGS and GT5 cells, the transfected cells were sorted by flow cytometry and then confirmed by western blot as Figure 5. We found that knock down *RhoA* significantly decreased YAP1 expression in both AGS and GT5 cells (Figure 5).

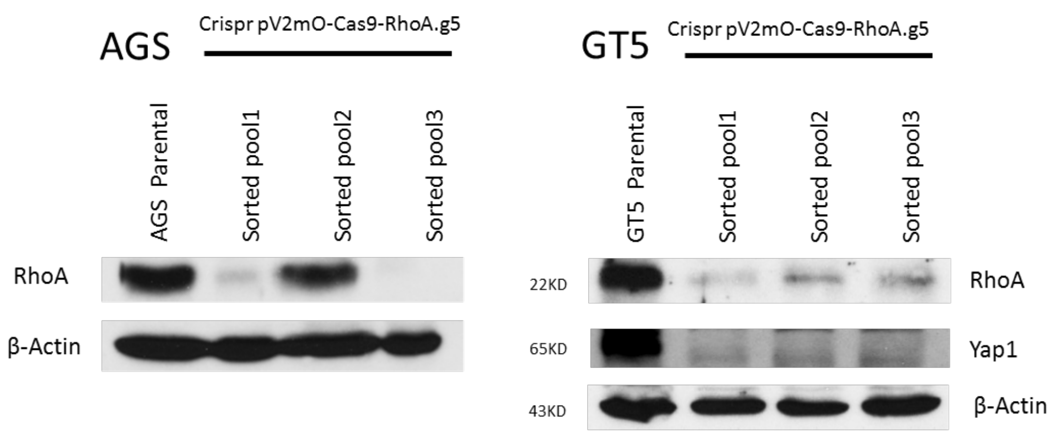


Figure 5. Western blots confirm that RhoA successfully knockdown by expressing mOrange and Puro-resistant cells in both AGS and GT5 cells compared to parental cells. In GT5, RhoA KO also leads to Yap1 downregurated, suggesting RhoA is upstream of Hippo pathway coactivator Yap1 which may drive GAC tumorigenesis.

Findings of this Aim2 in this period: Our IDEA is on the right track. We have successfully inserted a fluorescent marker mOrange into MIT's Dr. Zhang's pLenti-Crispr-v2, making transfection into mammalian cells easier and visible under fluorescent microscope, it the same time, those cells under Crispr editing are also selectable with puromycin. 3) We have successfully knocked-out RhoA expression in cell lines of AGS and GT5. 4) With RhoA

knockout in cell line GT5, Yap1 is also significantly downregulated. 5) Reintroducing mutant *RhoA*-Y42C is in progress.
<u>2-3. *Establish RhoA hotspot mutations (Y42C and E64) in 293T and GAC cells and determine the association of RhoA mutations with Hippo signaling.*</u> To overexpress RhoA WT or mutated form in GAC cell lines, the *RhoA* gene with WT or mutation was inserted in-frame into pcDNA3.1HisB/V5 vector using the following primers.

hRhoA.Wt.F.Kpn1   5’ aaGGTAC
CATGGCTGCCATCCGGAAGAAACTGGTGATTGTTGGTGATG 3’
hRhoA.Wt.R.Xba1   5’ aaTCTAGActCAAGACAAGGCACCCAGATTTTTTCTTCCCACG 3’

The following primers were used to generate mutational Y42C or E64 *RhoA* gene to insert into the vector. The point mutation Y42C or E64 of RhoA is achieved by overlaying PCR products of Wt.F-Y42C.R and Y42F-Wt.R. PCR products are amplified by NEB (New England BioLab)’s high fidelity Q5 polymerase. The final WT and Y42C *RhoA* genes were verified by sequencing.

hRhoA.Y42C.F   5’
GTGCCCACAGTGTTTGAGAACTCTGTGGCAGATATCGAGGTGGATG 3’
hRhoA.Y42C.R   5’
CATCCACCTCGATATCTGCCACAGAGTTCTCAAACACTGTGGGCAC 3’

These following qPCR primers will be used to access mRNA level upregulation.
hRhoA.mRNA.F   5’ GTCTGGTCTTCAGCTACCCG 3’
hRhoA.mRNA.R   5’ AGGCTCCATCACCAACAATC 3’

As shown in Figure 6, we have successfully transfected either *RhoA* WT or mutation into 293T cells and found that YAP1 and its target SOX9 and CTGF and C-MYC significantly upregulated especially by mutation of *RhoA* in 293T cells.
Similarly, we transfected *RhoA* WT or Y42C and E64 mutation into AGS GAC cell line and also found that *RhoA* mutation can decrease phosphor-Lats1/2 and p-MST1/2 which are tumor suppressors and upstream of YAP1 and increased its target CTGF (Figure 7).

Figure 6. Mutation of *RhoA* in 293T cells activate YAP1 and its down stream targets by Western blot(A) and Q-PCR(B)

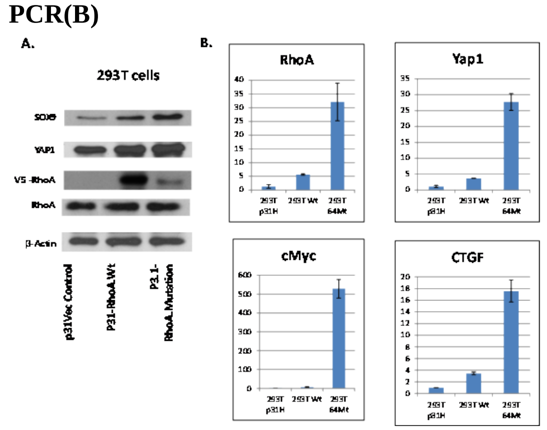
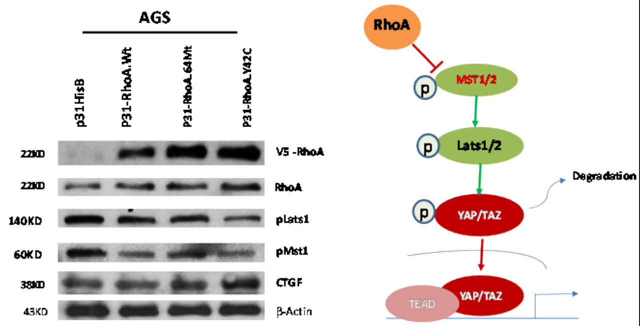


Figure 7. Expression of mutations or WT RhoA in AGS GAC cell lines decreased phosphor-MST1/2 and phosphor-Lats1/2 and increased YAP1 target CTGF.



3. Develop a genetic mouse model of RhoA mutation

We are establishing an *in vivo* RhoA Y42C mutation knock out in mouse model in the gastroesophageal region. The figures 8 and 9 show our constructs and plasmid.

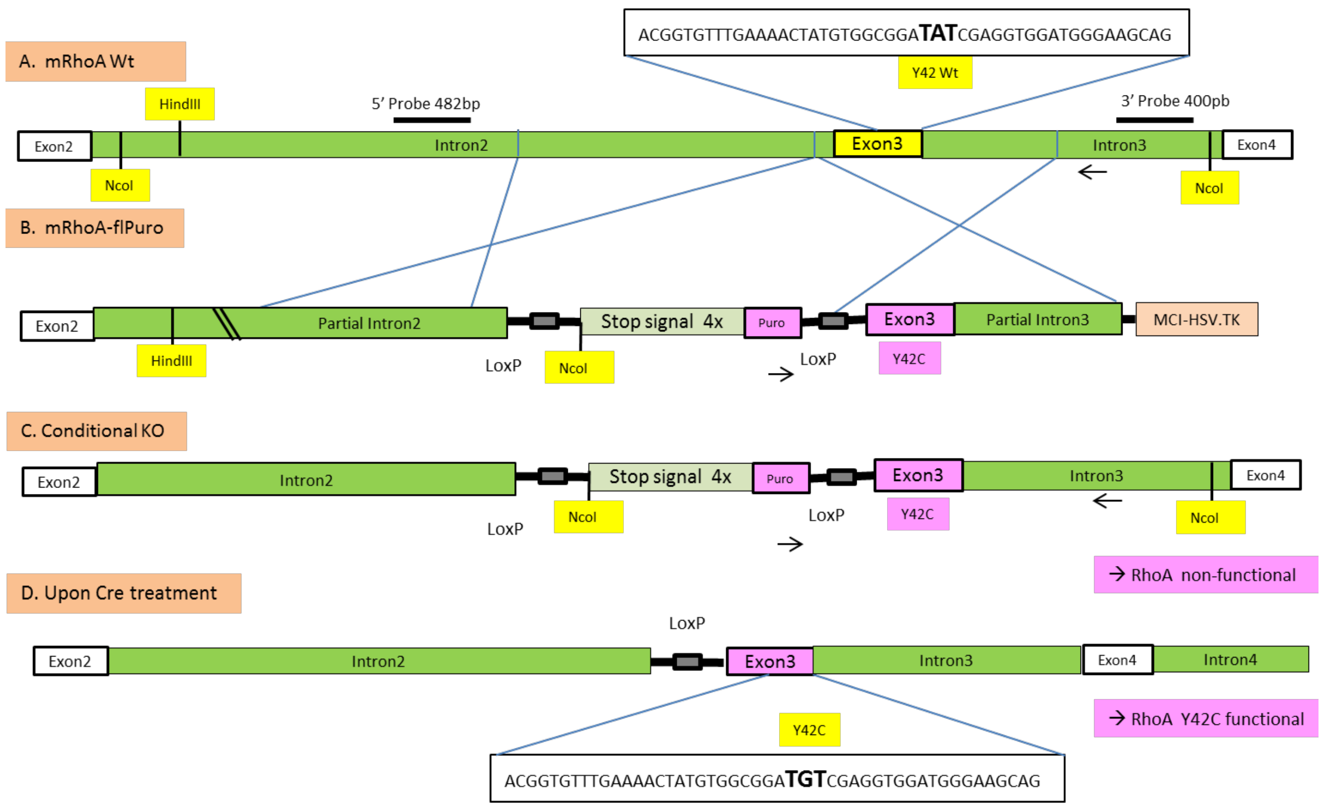


Figure 8. Schematic strategy of constructing conditional *RhoA* knock-out and Y42C knock-in mouse model (mRhoA-5'Flank- LSL- mRhoA-3'Flank- MCI-HSV.TK-pL253).

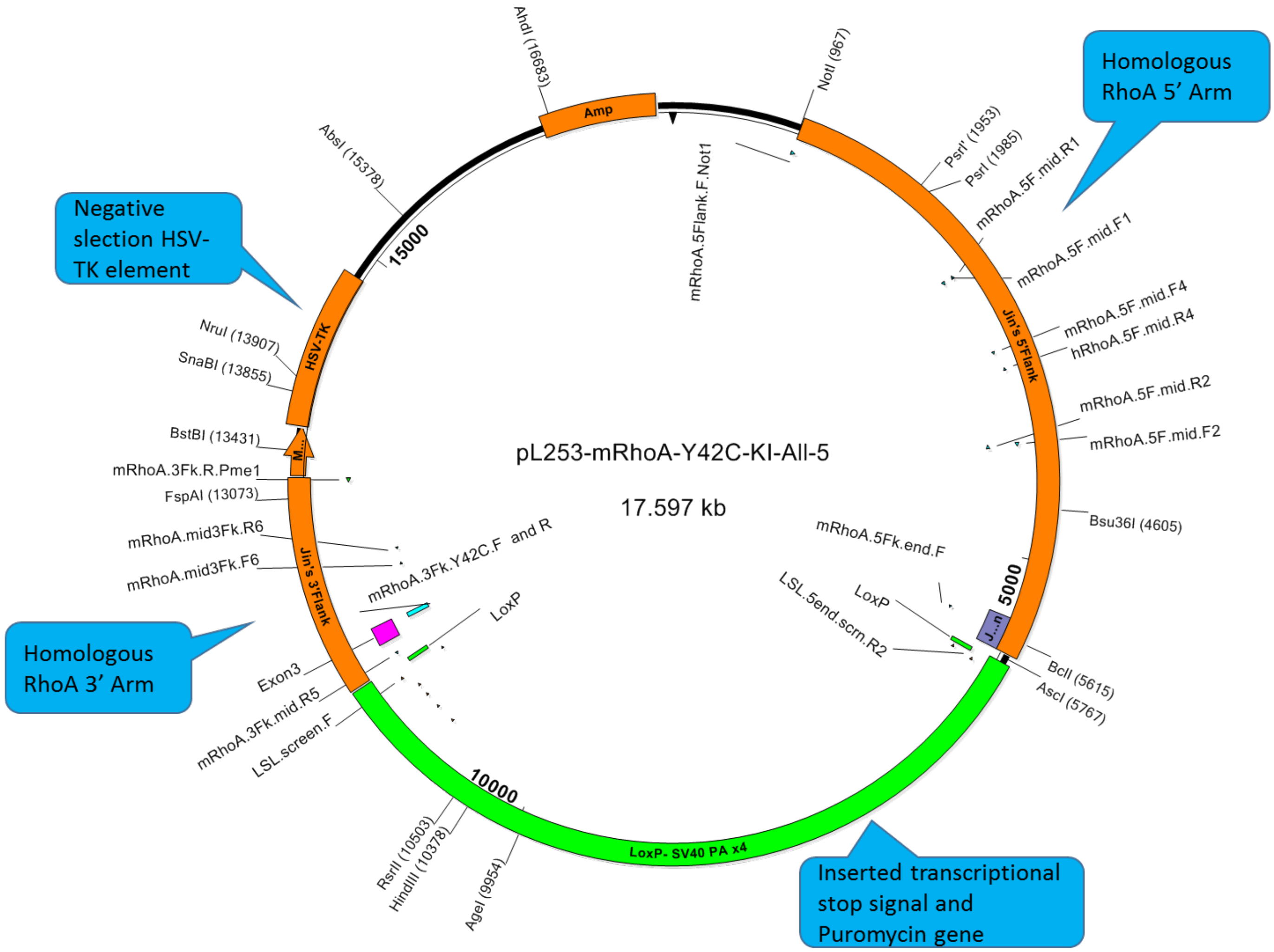


Figure 9. A knock-out and Y42C knock-in plasmid based on the above schematic strategy of homologous integration is constructed, with all the elements verified. The plasmid is linearized by Not1 digestion, before it is electroporated into mouse ES F1 cells.

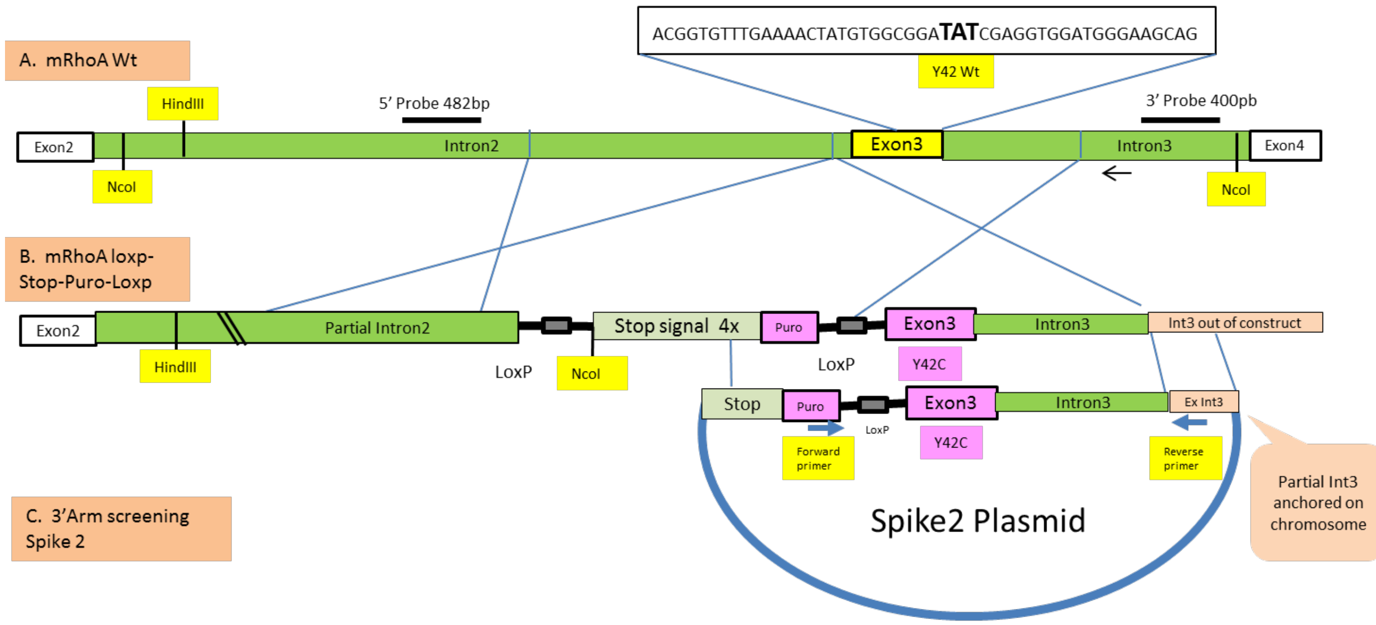
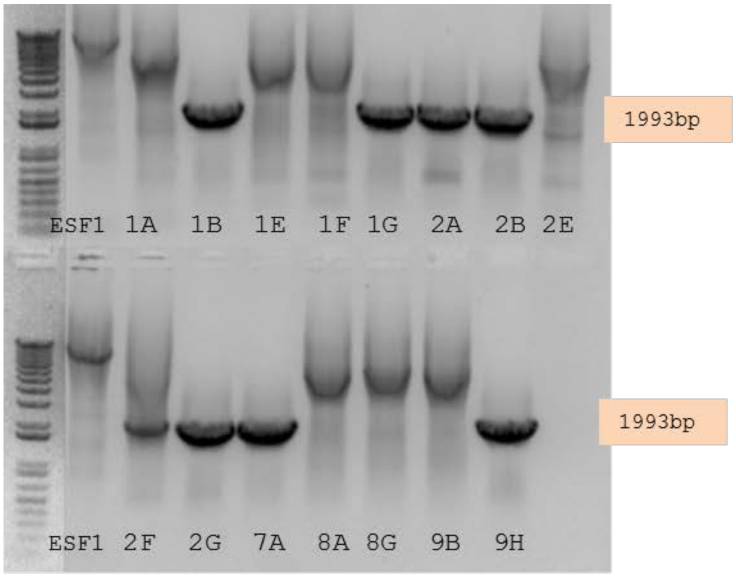


Figure 10. For PCR screening of our construct homologously integrated into *RhoA* gene, a mock plasmid Spike2 is constructed, with Forward primer anchored on LSL-Puro (Loxp-Stop-Puro-Loxp) insert, the Reverse primer anchored out of the 3'Arm, but on Chromosome Intron3.

GEMF (Genetically Engineered Mouse Facility) of Univ Texas MDACC, had electroporated murine ES F1 cells with the Not1-linearized plasmid (pL253-mRhoA-Y42C-All5-Not1) of Figure 12, ESF1 cells were screened with Puromycin 4ug/ml for 2 weeks, surviving clones were propagated and extracted genomic DNAs. Screening is to find the clones of *RhoA*-Y42C construct integrated in-situ onto *RhoA* locus by homologous recombination. Preliminary screening of partial Puromycin fragment presence in clones showed most of the clones are positive with Puromycin integration.

Progress of the first funding year in Aim3: 1) *In-vivo* murine model of construct of mRhoA-5'arm-LoxP-4xTranscriptional stop signal-Puro-LoxP-RhoA.Exon3.Y42C-3'arm was successfully constructed and verified by sequencing and digestion; 2) The construct was transfected into cell lines AGS and GT5, both cell lines conferred Puro resistance and are detectable by PCR method; 3) A spike to mimic in-situ homologous integration of the construct into *RhoA* Exon3 was created, and long PCR detection of the 3' arm with one primer anchored on chromosome out of 3' arm was successful; 4) Murine ESF1 cells electroporated with the above Knockout construct (pL253-mRhoA-Y42C-All5-Not1), propagated and extracted genomic DNAs, and partial Puro gene is detected in most clones, further long PCR screening will ensue.



10/11/17 Roche ELT nested PCR in 25ul, 2’00”ext, 57C anneal, 8+25 cyc. 2nd PCR (LSL.scrn.F2—mRhoA.3Fk.scrn.R2, 1993bp) from 1st PCR (LSL.scrn.F4—mRhoA.3Fk.scrn.R, 2364bp) as templates. Left columns are from ESF1 cells 4000 copies/25ul. Load 15ul.

Figure 11. Preliminary screening of *RhoA*-Wt-KO and *RhoA*-Y42C-KI in the 3’ arm with one primer anchored on chromosome out of construct and the other on LSL (LoxP-transcriptional stop-LoxP), showed there are at least 8 clones of ES F1 clones are positive, which indicates that the construct has integrated in situ on the position of Exon2 of the RhoA gene. Further screening and verification is pending.

Findings of Aim3 in this funding period: 1) *In-vivo* murine model of construct of mRhoA-5’arm-LoxP-4xTranscriptional stop signal-Puro-LoxP-RhoA.Exon3.Y42C-3’arm was successfully constructed and verified by sequencing and digestion; 2) The construct was transfected into cell lines AGS and GT5, both cell lines conferred Puro resistance and are detectable by PCR method; 3) A spike to mimic in-situ homologous integration of the construct into RhoA Exon3 was created, and long PCR detection of the 3’ arm with one primer anchored on chromosome out of 3’ arm was successful; 4) Murine ESF1 cells electroporated with the above knockout construct (pL253-mRhoA-Y42C-All5-Not1), propagated and extracted genomic DNAs, and partial Puro gene is detected in most clones; 5) Further long PCR screening of the 3’arm of homologous in-situ integration of RhoA-Wt-KO and RhoA-Y42C-KI proved that at least 8 clones are positive.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

We have Dr. Kazuto Harada (partially supported by grant from Japan) and masters student from Brazil (Melissa Pizzi). Both of these individuals have learned from the experiments performed to date. They will remain in our lab for another year and will grow professionally.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Results are not final and have not been distributed yet

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are working on all 3 aims to finish experiments as proposed.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We are very pleased that the IDEA we proposed (that RhoA mutation regulate Yap1) appears to be true. When we knocked down the RhoA mutation, the Yap1 levels were consistently low. This is very encouraging.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

RhoA mutations are rare and limited to a few tumor types; however, Yap1 is often involved in various cross talks and confers resistance to therapy. We believe that our final results will produce greater understanding of Yap1 for other researchers engaged in other tumor types.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We mentioned in our report that we had planned to perform RhoA staining in our GAC TMA, however, after noticing that the current antibody cannot distinguish between WT and mutated RhoA protein, we are not looking for an antibody that can distinguish.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  No publications yet but we are planning.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

None

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

None

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

None

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

None

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

N/A

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Shumei Song*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*125784*
Nearest person month worked:	*3*
Contribution to Project:	*No Change*
Funding Support:	*N/A*

Name:	*Jiankang Jin*
Project Role:	*Technician*
Researcher Identified:	*161357*
Nearest person month worked:	*12*
Contribution to Project:	*No Change*
Funding Support:	*N/A*

Name:	*Ailing Scott*
Project Role:	*Research Data Coordinator*
Researcher Identified:	*142319*
Nearest person month worked:	*2*
Contribution to Project:	*No Change*
Funding Support:	*N/A*
Name:	*Kazuto Harada*
Project Role:	*PostDoc*
Researcher Identified:	*224936*
Nearest person month worked:	*3*
Contribution to Project:	*No Change*
Funding Support:	*N/A*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Dr. Ajani – 3 changes, indicated by italics
Dr. Song – 3 changes, indicated by italics
Dr. Johnson – no changes

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)
- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

Previous/Current/Pending Support for DOD

For all **previous (award period of performance ending within the past 5 years), current, and pending research support,** include the title, time commitments, supporting agency, name and address of the funding agency's procuring Contracting/Grants Officer, performance period, level of funding, brief description of the project's goals, and list of the specific aims. If applicable, identify where the proposed project overlaps with other existing and pending research projects. Clearly state if there is no overlap. If there is no previous, current, or pending support, enter "None." An updated previous, current, and pending support document will be required if an award is recommended for funding. Please note there is no page limit. Add additional pages as deemed necessary.

Ajani, Jaffer

PREVIOUS (for the past 5 years)

Title:	Prediction of Pathologic Complete Response by Gene Expression Profiling in Esophageal Adenocarcinoma
Effort:	0.96 calendar months
Supporting Agency:	National Cancer Institute
Grants Officer:	Magdalena Thurin, 9609 Medical Center Dr. Rockville, MD 20850
Performance Period:	02/01/2010-01/31/2016
Funding Amount	1,509,813
Project Goals	To individualize therapy based on molecular biology for patients with esophageal cancer and pave the way for a strategy in the future that will allow administration of effective therapy, improve safety, and preserve the esophagus in some patients.
Specific Aims	SA 0: (Additional discovery) Conduct gene expression profiling in ~ 73 tri-modality (TM) esophageal cancer patients to reach the proposed 120 patients (Funded: R21CA127612) to derive a gene list correlating with three outcomes by pathologic subgrouping (add data to Specific Aim 1C) SA 1: Conduct gene expression profiling in independent 120 TM esophageal cancer patients and establish a large discovery (training) cohort. A. Prospectively collect clinical data and tissue in 60 TM patients. B. Conduct gene expression profiling in 120 TM patients to validate the gene list derived from Aim 0 C. Combine data from Specific Aims 0 and 1B to build a robust training set (n=240) to identify ~100 best performing genes correlating with three outcomes by pathologic subgrouping. SA 2: Validate ~100 gene signature derived in Specific Aim 1C in 100 TM esophageal cancer patients (50 derived from Specific Aim 1 and 50 prospectively) to narrow the gene list to ~10 genes A. Prospectively collect clinical data and tissue in 50 TM patients. B. Validate ~100 genes derived from SA 1 by micro-fluidic card technology in various cohorts: 50 patients from Specific Aim 1 and 50 prospective patients to finalize the number of genes to ~10 correlating with three outcomes by pathologic subgrouping for prospective validation. SA 3: Validation of the ~10 gene signature in prospectively accrued 60 TM esophageal cancer patients. A. Prospectively collect clinical data and tissue in 60 TM patients. B. Validate ~10-gene signature derived from Specific Aim 2B by Real Time PCR in a

	prospectively cohort of 60 TM patients to establish that it has high level of specificity (>/= 80%) and reasonable level of sensitivity (>/=45%).
Overlap:	None
Title:	Development and validation of prognostic model for gastric cancer
Effort:	0.36 calendar months
Supporting Agency:	National Cancer Institute
Grants Officer:	Magdalena Thurin, 9609 Medical Center Dr. Rockville, MD 20850
Performance Period:	04/01/2010-01/31/2014
Funding Amount	$186,750
Project Goals	The goal of this proposal is to find and validate new prognostic biomarkers for gastric cancer.
Specific Aims	1. Validation of 6-gene-based risk score by conducting real-time qRT-PCR experiments in independent Asian GC patients cohort (n=168). 2. Validate the association of gene expression patterns with prognosis in independent Western cohorts. 3. Improvement of risk score by combining preliminary and new gene expression data and validation of improved risk score in prospective patient cohort.
Overlap:	None
Title:	Genetic instability & risk for esophageal carcinoma.
Effort:	0.6 calendar months
Supporting Agency:	National Cancer Institute
Grants Officer:	Schully, Sheri D, 6100 Executive Blvd Room 2B03, MSC 7523 Bethesda, MD 20892
Performance Period:	03/10/2008 – 01/31/2014
Funding Amount	$2,860,710
Project Goals	The major goal of this project is to build a case-control study to investigate risk factors for esophageal adenocarcinoma (EAC) and to further our understanding on EAC tumorigenesis.
Specific Aims	1. Assess markers of genetic instability in surrogate tissue (lymphocytes) We will test the hypothesis that: 1) individuals with shortened telomeres are at greater risk for EAC than those who have long telomeres; 2)adverse genotypes of the telomere length maintenance pathway are associated with an increased risk for EAC; 3) cancer cases show increased levels of mutagen-induced DNA damage in PBLs compared with controls. 4) the finding of adverse genotypes in DNA repair genes is associated with an increased risk for EAC. 2. Assess genotype-phenotype associations from markers of susceptibility We will test the hypothesis that: 1) the adverse genotypes of telomere length maintenance pathway will predict telomere dysfunction; 2) the adverse genotypes of the NER pathway will predict higher levels of BPDE-induced DNA damage and that the adverse genotypes of the BER and DSB pathways will predict higher levels of γ-radiation induced DNA damage. 3. Correlate markers in surrogate (lymphocytes) and target (tumor) tissue We will test the hypothesis that individuals with short telomeres, adverse genotypes, and/or high levels of mutagen-induced DNA damage are at higher risk for genetic alterations in the target tissue.
Overlap:	None

Title:	*CDK Inhibitors as Adjunctive to 5-FU and/or Radiation in Esophageal Adenocarcinoma – Assessment of Efficacy and Predictive Biomarkers.*
Effort:	*0.12 calendar month*
Supporting Agency:	*Cancer Prevention & Research Institute of Texas*
Grants Officer:	*Michael Brown, 1701 North Congress Avenue, Ste 6-127, Austin, TX 78701*

Performance Period:	*09/01/2014 – 08/31/2017*
Funding Amount	*$882,133*
Project Goals	*To evaluate efficacy and mechanism of action of CDK inhibitor in vitro and in vivo that we can have strong data to support a clinical trial in the future.*
Specific Aims	*Aim 1: (1a) In vitro evaluation of cytotoxic effects of clinically used CDK inhibitors with CDK9 inhibitory activities, alone and in combination with 5-FU and radiation on EAC. 1(b) In vitro studies assessing influence of alteration in MCL-1 on the efficacy of CDK inhibitors, 5-FU, radiation and combination of these agents on EAC. 1(c) Understand mechanisms regulating MCL-1 alterations induced by 5-FU, radiation and CDK inhibitors in EAC cell lines in vitro.* *Aim 2: 2(a) In vivo evaluation of efficacy of chosen CDK inhibitor; alone an in combination with 5-FU and radiation; in 5-FU or radiation naïve and 5-FU or radiation resistant xenografts of EAC (2b) In vivo evaluation of efficacy of chosen CDK inhibitor; alone and in combination with 5-FU or radiation in patient derived xenografts (PDSx) or EAC. (2c) Assess role of MCL-1 and other CDK 9 targets as markers of 5-FU and/or radiation resistance in PDX, 5-FU or radiation naïve and resistance xenografts. Aim 3: Study the MCL-1 and other selected CDK9 targets as potential markers of chemoradiation resistance in EAC samples of patients who were treated with preoperative neoadjuvant therapy and surgery.*
Overlap:	*None*

CURRENT

Title:	Inhibition of Hedgehog Signaling in Gli-1+ Adenocarcinoma in Esophagus and GE
	Junction
Effort:	0.9 calendar month
Supporting Agency:	National Cancer Institute
Grants Officer:	Leslie Hickman, 9609 Medical Center Dr. West Tower, 2nd fl, Rockville MD 20850
Performance Period:	09/01/2013-08/31/2018
Funding Amount	830,985
Project Goals	To prospectively validate, in the CLIA lab, a validated 3-biomarker (Gli-1, Shh and ALDH1) predictive signature of response to chemo radiation in patients with EAC and to implement this signature in patients to customize therapy using Hh inhibitor GDC-0449.
Specific Aims	Aim 1: To conduct a phase IB/II trial of GDC-0449 (NSC 747691) plus preoperative chemoradiation in enriched patients with localized nuclear Gli-1+ EAC. A: Conduct a phase IB trial to establish safety of GDC-0449 plus chemoradiation. B: Conduct a phase II trial to estimate the rate of pathCR and establish pharmacodynamic effects of GDC-0449 (compare with historical controls). C: Carry out a prospective validation of 3-biomarker pathCR-predicting signature. Aim 2: To identify the molecular pathways of GDC-0449 drug resistance in cell lines and patients in Aim 1. A: Determine the change in activation and expression status of proteins in cell signaling pathways after GDC-0449 treatment and identify key molecules of GDC-0449 resistance (Cell lines and patients). B: Determine the change of gene expression profiles after GDC-0449 treatment and establish biomarkers for GDC response and resistance.

Title	Exploiting RhoA Mutations in Diffuse Gastric Adenocarcinoma and Targeting Intertwined RhoA and Yap1 Pathways for Therapeutic Advantage
Effort	0.6 calendar months
Supporting Agency	DOD
Grants Officer	Wendy A. Baker
Performance Period	9/15/2016 – 9/14/2018
Funding Amount	$640,000
Project Goals	To establish if mutated RhoA signaling activates Yap1 signaling in dGAC. We will inhibit both pathways for maximum advantage in preclinical dGAC genetic and xenograft models in vitro and in vivo.
Specific Aims	1) Identification of overexpression and/or activating mutations of RhoA in novel cancer models of GAC (patient derived primary tumor cells and GAC TMA) to determine their clinical relevance and relationship to Yap1 activation. 2) Elucidate oncogenic functions of WT/mutant RhoA in GAC cells and determine if WT or mutant RhoA activates Hippo pathways coactivator Yap1 by assessing nuclear accumulation and transcriptional activity (bidirectional) in GAC cells. 3) Develop a genetic mouse model of RhoA mutation an dYap1 overexpression in stomach and assess the effects of RhoA inhibitor or in combination of Yap1 inhibition in vitro an din vivo to set the stage for a clinical trial in dGAC patients.
Overlap	None
Title:	*CA160433: Immune-Suppression and Tumor-Stromal Interaction Mediated by Galextin-3 in Gastric Cancer – Implications of Novel Therapeutic Strategies*
Effort:	*0.12 calendar month*
Supporting Agency:	*Department of Defense*
Grants Officer:	*Jaclyn P. Svincek*
Performance Period:	*9/30/2017 – 9/29/2019*
Funding Amount	*$640,000*
Project Goals	*The goal is to understand Gal-3-mediated immune suppression and tumor-stromal interactions that could be exploited therapeutically.*
Specific Aims	*Aim 1: Determine the association between Gal-3 expression and immune checkpoint proteins PD-L1, CD47 and other immune factors in tumor tissue of GAC TMA and define their clinical relevance.* *Aim 2: Carry out functional studies to define if Gal-3 up-regulation of PDL-1 and CD47 in tumor cells and activation of TAF/TAM in the stroma.* *Aim 3: Determine the function of Gal-3 in tumor immunosuppression in Gal-3 deficiency mouse model and preclinical test the effects of Gal-3 inhibitor or in combination with anti-PD-L1 or inhibitors of CD47 and CSF1R on GAC tumor growth in vivo mouse model.*
Overlap:	*None*
Title:	*CA160445: Discover Novel Therapeutic Strategies for Peritoneal Metastases from Gastric Adenocarcinoma*
Effort:	*0.6 calendar month*
Supporting Agency:	*Department of Defense*
Grants Office:	*Jamie A. Shortall*
Performance Period:	*9/30/2017 – 09/29/2020*
Funding Amount:	*$459,000*
Project Goals:	*To identify novel therapeutic targets for gastric adenocarcinoma patients with peritoneal carcinomatosis by stydying cancer stem cell pathways and mutli-omics integrated analyses.*

Specific Aims:	*Aim 1: Molecular profiling of CSC pathways from PC.* *Aim 2: Identificaiton of additional novel therapeutic targets in human PC cells through a multi-omics platform.* *Aim3: Preclinical evaluations of targets identified in Aim 2.*
Overlap	*None*

PENDING

None

Previous/Current/Pending Support for DOD

For all **previous (award period of performance ending within the past 5 years), current, and pending research support,** include the title, time commitments, supporting agency, name and address of the funding agency's procuring Contracting/Grants Officer, performance period, level of funding, brief description of the project's goals, and list of the specific aims. If applicable, identify where the proposed project overlaps with other existing and pending research projects. Clearly state if there is no overlap. If there is no previous, current, or pending support, enter "None."

Song, Shumei

PREVIOUS

Title:	Dysfunctional of TGF-β signaling via β2SP activates SOX9 and C-MYC via Notch signaling in Hepatocellular Carcinoma
Effort:	No effort
Supporting Agency:	MDACC Internal Funding Cyrus Scholar Award- Endowment
Grants Officer:	Claudia Delgado, 1515 Holcombe Blvd, Houston, TX 77030
Performance Period:	07/01/2011-06/30/2014
Funding Amount	15,000
Project Goals	To explore how TGF- β switches its role during tumor initiation and progression by applying genetic β2SP+/-/Smad4+/- Mice model
Specific Aims	Dissect how this change happens or the drivers for this process are very important to find the cure for cancer. We hypothesize that an imbalance of stem cell signaling mediated by loss of TGF- β leads to activation of Notch in these tumors and drive initiation and progression of EC and HCC
Overlap:	None

Title:	Dysfunctional TGF-β Signaling with Constitutively Active Notch Signaling Contributes to Barrett's Esophageal Adenocarcinoma
Effort:	No effort
Supporting Agency:	MDACC Internal Funding
Grants Officer:	Claudia Delgado, 1515 Holcombe Blvd, Houston, TX 77030
Performance Period:	01/05/2012-01/04/2014
Funding Amount	49,984
Project Goals	The goal of this project is identify the mechanisms and predictors of Barrett's progression and yield novel therapeutics for esophageal adenocarcinoma
Specific Aims	Identify the mechanisms and predictors of Barrett's progression Yield novel therapeutics for esophageal adenocarcinoma
Overlap:	None
Title:	Prediction of Pathologic Complete Response by Gene Expression Profiling in Esophageal Adenocarcinoma
Effort:	2 calendar months
Supporting Agency:	National Cancer Institute
Grants Officer:	Magdalena Thurin, 9606 Medical Center Dr. Bethesda, MD 20892
Performance Period:	02/01/2010-01/31/2016
Funding Amount	300,665
Project Goals	To individualize therapy based on molecular biology for patients with esophageal cancer and pave the way for a strategy in the future that will allow administration of effective therapy, improve safety, and preserve the esophagus in some patients.

Specific Aims	SA 0: (Additional discovery) Conduct gene expression profiling in ~ 73 tri-modality (TM) esophageal cancer patients to reach the proposed 120 patients (Funded: R21CA127612) to derive a gene list correlating with three outcomes by pathologic subgrouping (add data to Specific Aim 1C) SA 1: Conduct gene expression profiling in independent 120 TM esophageal cancer patients and establish a large discovery (training) cohort. A. Prospectively collect clinical data and tussue in 60 TM patients. B. Conduct gene expression profiling in 120 TM patients to validate the gene list derived from Aim 0 C. Combine data from Specific Aims 0 and 1B to build a robust training set (n=240) to identify ~100 best performing genes correlating with three outcomes by pathologic subgrouping. SA 2: Validate ~100 gene signature derived in Specific Aim 1C in 100 TM esophageal cancer patients (50 derived from Specific Aim 1 and 50 prospectively) to narrow the gene list to ~10 genes A. Prospectively collect clinical data and tissue in 50 TM patients. B. Validate ~100 genes derived from SA 1 by micro-fluidic card technology in various cohorts: 50 patients from Specific Aim 1 and 50 prospective patients to finalize the number of genes to ~10 correlating with three outcomes by pathologic subgrouping for prospective validation. SA 3: Validation of the ~10 gene signature in prospectively accrued 60 TM esophageal cancer patients. A. Prospectively collect clinical data and tissue in 60 TM patients. B. Validate ~10-gene signature derived from Specific Aim 2B by Real Time PCR in a prospectively cohort of 60 TM patients to establish that it has high level of specificity (>/= 80%) and reasonable level of sensitivity (>/=45%).
Overlap:	None

CURRENT

Title:	Inhibition of Hedgehog Signaling in Gli-1+ Adenocarcinoma in Esophagus and GE Junction
Effort:	1 calendar month
Supporting Agency:	National Cancer Institute
Grants Officer:	Leslie Hickman, 9609 Medical Center Dr. West Tower, 2nd fl, Rockville MD 20850
Performance Period:	09/01/2013-06/30/2018
Funding Amount	279,793
Project Goals	To prospectively validate, in the CLIA lab, a validated 3-biomarker (Gli-1, Shh and ALDH1) predictive signature of response to chemo radiation in patients with EAC and to implement this signature in patients to customize therapy using Hh inhibitor GDC-0449.
Specific Aims	Aim 1: To conduct a phase IB/II trial of GDC-0449 (NSC 747691) plus preoperative chemoradiation in enriched patients with localized nuclear Gli-1+ EAC. A: Conduct a phase IB trial to establish safety of GDC-0449 plus chemoradiation. B: Conduct a phase II trial to estimate the rate of pathCR and establish pharmacodynamic effects of GDC-0449

	(compare with historical controls). C: Carry out a prospective validation of 3-biomarker pathCR-predicting signature. Aim 2: To identify the molecular pathways of GDC-0449 drug resistance in cell lines and patients in Aim 1. A: Determine the change in activation and expression status of proteins in cell signaling pathways after GDC-0449 treatment and identify key molecules of GDC-0449 resistance (Cell lines and patients). B: Determine the change of gene expression profiles after GDC-0449 treatment and establish biomarkers for GDC response and resistance.
Overlap:	None
Title:	CA150334: Exploiting RhoA Mutations in Diffuse Gastric Adenocarcinoma and Targeting Intertwined RhoA and Yap1 Pathways for Therapeutic Advantage
Effort:	25%
Supporting Agency:	DOD
Grants Officer:	Jamie A. Shortall
Performance Period:	10/01/2016 – 09/30/2018
Funding Amount	$640,000
Project Goals	The goal is to show that the RhoA and Yap1 pathways are novel targets for dGACs and the dual inhibition will provide added advantage against dGACs.
Specific Aims	Aim 1: Identification of overexpression and/or activating murations of RhoA in novel cancer models of GAC (patient derived primary tumor cells and GAC TMA) to determine their clinical relevance and relationship to Yap1 activation. Aim 2: Elucidate oncogenic functions of WT/mutant RhoA in GAC cells and determine if WT or mutant RhoA activates Hippo pathway coactivator Yap1 by assessing nuclear accumulation and transcriptional activity (bidirectional) in GAC cells. Aim 3: Develop a genetic mouse model of RhoA mutation and Yap1 overexpression in stomach and assess the effects of RhoA inhibitor or in combination of Yap1 inhibition in vitro and in vivo to set the stage for a clinical trial in dGAC patients.
Overlap:	None
Title:	*CA160445: Discover Novel Therapeutic Strategies for Peritoneal Metastases from Gastric Adenocarcinoma*
Effort:	*15%*
Supporting Agency:	*DOD*
Grants Officer:	*Jamie A. Shortall*
Performance Period:	*09/30/2017 – 9/29/2020*
Funding Amount	*$734,399*
Project Goals	*The goal of this project is to identify novel therapeutic targets for gastric adenocarcinoma (GAC) patients with peritoneal carcinomatosis (PC) by studying cancer stem cell (CSC) pathways and multi-omics integrated analyses.*
Specific Aims	*Aim 1: (1a) Expand PC cells by in vitro conditional reprogramming of cells (CRCs) and cell line generation. 1(b) Establish PC-PDX model and spontaneous-PC-PDX model. 1(c) Study functional relevance of CSC pathways in in vitro/in vivo.* *Aim 2: (2a) RNA/Exome Seq profiling (2b) Proteomic/exosome characterization (MS). (2c) Profiling miRNAs/IncRNAs* *Aim 3: (3a) Genetic modulations of the candidate targets in human PC cells emergin from Aim 2 in vitro to establish their functional relevance. (3b) In vitro studies of human PC cells with novel agents against CSC pathways and other novel targets emerging from Aim 2. (3c) In vivo studies with human PC PDX models.*
Overlap:	*None*

Title:	*CA160433: Immune-Suppression and Tumor-Stromal Interaction Mediated by Galextin-3 in Gastric Cancer – Implications of Novel Therapeutic Strategies*
Effort:	*0.12 calendar month*
Supporting Agency:	*Department of Defense*
Grants Officer:	*Jaclyn P. Svincek*
Performance Period:	*9/30/2017 – 9/29/2019*
Funding Amount	*$640,000*
Project Goals	*The goal is to understand Gal-3-mediated immune suppression and tumor-stromal interactions that could be exploited therapeutically.*
Specific Aims	*Aim 1: Determine the association between Gal-3 expression and immune checkpoint proteins PD-L1, CD47 and other immune factors in tumor tissue of GAC TMA and define their clinical relevance.* *Aim 2: Carry out functional studies to define if Gal-3 up-regulation of PDL-1 and CD47 in tumor cells and activation of TAF/TAM in the stroma.* *Aim 3: Determine the function of Gal-3 in tumor immunosuppression in Gal-3 deficiency mouse model and preclinical test the effects of Gal-3 inhibitor or in combination with anti-PD-L1 or inhibitors of CD47 and CSF1R on GAC tumor growth in vivo mouse model.*
Overlap:	*None*

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.